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Rec'd PCT/PTO 27 DEC 2004

INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 10.216/K-TMA3	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/HU 03/00044	International filing date (day/month/year) 17.06.2003	Priority date (day/month/year) 25.06.2002
International Patent Classification (IPC) or both national classification and IPC C12M1/00		
Applicant SZEKERES, György et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
- These annexes consist of a total of 11 sheets.

3. This report contains indications relating to the following items:
- I ☒ Basis of the opinion
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☐ Certain observations on the international application

Date of submission of the demand 15.01.2004	Date of completion of this report 26.08.2004
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Steinheimer-Breitkre Telephone No. +49 89 2399-7115 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/HU 03/00044

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-7 received on 26.06.2004 with letter of 24.06.2004

Claims, Numbers

1-3 received on 26.06.2004 with letter of 24.06.2004

Drawings, Sheets

1/3-3/3 received on 26.06.2004 with letter of 24.06.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/HU 03/00044**

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-3
	No: Claims	
Inventive step (IS)	Yes: Claims	1-3
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-3
	No: Claims	

2. Citations and explanations

see separate sheet

V Reasoned statement

V.1 Documents cited

Reference is made to the following documents:

- D1: WO 01 51910 A (MENGEL MICHAEL ;LILISCHKIS RICHARD (DE); WASIELEWSKI REINHARD (DE)) 19 July 2001 (2001-07-19)
- D2: US-A-5 002 377 (BATTIFORA HECTOR A ET AL) 26 March 1991 (1991-03-26) cited in the application
- D3: US-A-6 103 518 (LEIGHTON STEPHEN B) 15 August 2000 (2000-08-15) cited in the application
- D4: US-B1-6 383 801 (LEIGHTON STEPHEN B) 7 May 2002 (2002-05-07)
- D5: WO 01 98525 A (CLINOMICS LAB INC ;MURACA PATRICK J (US)) 27 December 2001 (2001-12-27)
- D6: WO 99 44063 A (US HEALTH ;KONONEN JUHA (US); KALLIONIEMI OLLI (US); LEIGHTON STEP) 2 September 1999 (1999-09-02)
- D7: WO 01 22086 A (COHEN JONATHAN) 29 March 2001 (2001-03-29)
- D8: WO 01 42796 A (SAUTER GUIDO ;KAKAREKA JOHN WILLIAM (US); SALEM GHADI HAMD I (US);) 14 June 2001 (2001-06-14)
- D9: WO 03 044213 A (GENENTECH INC) 30 May 2003 (2003-05-30)
- D10: US 2003/017446 A1 (CHU SUNNY WAI KEUNG ET AL) 23 January 2003 (2003-01-23)

V.2 Novelty, Inventive Step and Industrial Applicability (Art. 33 PCT)

- 2.1 The present invention concerns a tissue micro-array (TMA) building manual set, which includes a tissue core punch extractor and a TMA block mould, which has a lower and upper part according to claim 1. Said device has not been described in the prior art as represented by D1-D8. **Claims 1-3 are therefore considered to be novel.**
- 2.2 D1 describes a TMA block mould, which is used with a tissue core punch extractor (p. 6). D1 is considered to represent the closest prior art. The present application is different from D1 in that the TMA block mould does not have a lower and upper part but is made of one piece.
The technical effect of the mold having a lower and upper part is that the paraffin block holder is fixed to the empty paraffin block in one step when moulding the paraffin block. Therefore, there is no need to reheat the moulded and solidified

paraffin block part.

The problem underlying the application may be regarded as providing a device for making tissue micro-arrays in a single production step, i.e. without need for reheating.

The prior art does not suggest the solution described in claims 1-3. Claims 1-3 do therefore appear to involve an inventive step

2.3 All claims are considered to be industrially applicable.

V.3 The Applicant should also consider the following objections:

- 3.1 The application has been amended during the international phase. Information has been added to the description that has no basis in the application as originally filed, i.e. the discussion on the disadvantages of known procedures on p. 3-4 and the discussion of the advantages of the device of the invention on p. 5 and 7. These amendments therefore contravene Art. 19 (2) PCT.
- 3.2 Claims 1-3 contain references to the description and the drawings (numbers). According to Rule 6.2(a) PCT, claims should not contain such references except where absolutely necessary, which is not the case here.
- 3.3 D9 and D10 are intermediate documents and should be considered in the European regional phase.



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Tissue micro-array building manual set

The object of our invention is a tissue micro-array (TMA) preparing manual apparatus set, which includes a tissue core punch extractor and a TMA block mould with lower and upper parts.

The tissue micro-array preparing manual apparatus set is suitable for making TMA series, which enables the fast and accurate "home-made" preparation of TMA series well-fitted to the traditional, routine histology laboratory conditions.

TMA technology can be applied for the determination of diagnostic markers, as well as, parameters on the aggressiveness and other features of malignant tumors.

TMA is very useful for retrospective studies, i.e. for the investigation of thousands of molecular biological markers in thousands of histological specimens stored in pathology laboratories.

Similar to the DNA-chip, which allows the analysis of the genome mutations and abnormal gene expressions, the tissue micro-arrays enable the parallel processing of biological specimens. By using only one TMA, researchers have the opportunity for simultaneous comparative examination of various molecular parameters (DNA, RNA molecular biological techniques and immunohistochemistry/immunocytochemistry of antigens) in histological specimens of several malignant tumor tissues.

Numerous monoclonal antibodies, which determine the genome and phenotype of intact and pathological (mainly malignant) cells, and are specific for novel antigen-determinants, are being developed and introduced for diagnostic and therapeutic applications (immunomorphology). Diagnostic measures based on genetic methods, which can identify certain segments of DNA and/or RNA, are increasingly used (molecular biology). Several laboratories are involved in evaluating their tissue reactivity and potential applicability in the most prevalent tumors.

As a consequence, this diagnostic technique indeed seems to be widely used.

In the diagnostic technique, histology specimens from different tumor tissues are embedded in properly arranged sets into paraffin for the preparation of TMA. The TMA block is cut giving the various tumor tissues as point-like items in the histological light microscopic sections. Since each TMA block may render hundreds of parallel, 3-4 μ m thick serial sections,

hundreds of molecular parameters can be examined on the same micro-array. Several tumor markers can be analyzed within weeks, while this would take months per tissue specimen using traditional method.

According to our current knowledge, reactions made on standard-size histological sections (from frozen and/or fixed-embedded tissues) and cytological samples (smears, sediments, imprints, cytocentrifugates, etc.) are useful for the identification of molecular markers of tumors and for the prediction of disease progression. At the same time, merely limited number of samples can be examined due to technical and financial reasons, the evaluation is time-consuming, and comparative, as well as, reproducible examinations among different laboratories are difficult to carry out. First of all, the lack of reproducibility is the disadvantage of the so-called "multi-tissue block" technique that contains parts from various tissues/blocks in a simple histological block. Moreover, the distribution of individual molecular markers within the malignant tissue is often quite heterogeneous and, thus, a minute tissue part does not properly represent the biological features of the entire tumor cell population.

Application of the technique reminiscent of or rather based on the "chip" technology, i.e. the tissue micro-arrays, enables the investigation of hundreds or thousands of tissue specimens.

The knowledge in the field of medical biotechnological basic research is being multiplied, and its employment confirms both diagnostic and therapeutic measures. Nevertheless, the related applied research, research and development (R&D), as well as, clinical testing processes require years or even a decade and proportional financial means. Our invention, the TMA building manual set shortens the necessary testing phase in an accurate and cost-efficient way.

The information in the No. 4.820.504 US patent represents a well-known and widely used procedure, which serves for the preparation of a sample containing multiple tissue specimens. Its advantages include the simple procedure that can be applied in almost every laboratory equipped with routine and research facilities, low costs, and no need for procurement of new devices. However, the disadvantage of the technique is that the consecutive, side by side embedded tissue samples are randomly situated, not organized into lines and columns, which makes the evaluation rather difficult, the automatic (image analyzing) processing almost impossible or complicated and slow; the size of the individual tissue samples are merely roughly similar to each other. New instrument does not belong to the description of the procedure.

Our procedure and the object of our invention aims at addressing these disadvantages.

The No. 5.002.377 US patent is an instrument, which organizes tissue samples into regular lines and columns, and so gives a multiple tissue block. Its patent holder is the same as that of the previous instrument who is a well-known expert on this field, despite this technique has not become widely used. Its obvious advantage is that application of this instrument enables the preparation and embedding of regular, almost identical size tissue samples. Nevertheless, its disadvantage lies in that the individual tissue columns may be, though only slightly, shifted during block preparation. The largest disadvantages of the instrument are: its incompatibility with the most frequently applied and best known laboratory instruments (devices and semi-fixed assets) and, on the other hand, the need for three additional tools and the significant manual skills for their application. The first instrument includes multiple parallel cutting plates, which can cut out tissue columns from tissue samples, but can be applied exclusively on larger specimens. The other two components make possible the embedding of cut tissue columns into a single paraffin block.

The No. 6.103.518 US patent is a table device giving useful tissue micro-arrays. The procedure is based on the well-oriented technique of the recipient places of the paraffin block and the sampling from the selected sites of donor blocks (tissue cores). Besides its aforementioned advantages, its high price, complicated mechanical settings, and the need for one-by-one preparation of recipient holes of the recipient block represent serious drawbacks in the daily practice.

Publication document number WO 01/51910 presents a simple paraffin block construction procedure that can be used for the production of tissue micro-array series in which a mould that consists of several elements has been formed. On using the mould a basic body is moulded that has regularly arranged openings into which the tissue samples are then placed. Following this the basic body containing the tissue samples is heated up to the softening temperature limit in the interest of the basic body being able to combine with the liquid paraffin poured onto one of the surfaces in the following phase of the procedure and so form a single unit with it. This liquid paraffin layer serves to assist the fixing of the paraffin block holder to the paraffin block containing the cylindrical holes and the tissue samples contained in them.

However, a significant disadvantage of this solution is that the paraffin block that contains the cylinders with the tissue samples in them is subjected to a significant heat effect. Due to the heat effect and the re-softening of the paraffin the tissue cylinders move, which can cause

problems when making the sections. Another disadvantage of the subsequent heat effect is that the amount of heat required to soften the paraffin may damage the tissue sample cylinders, which may cause irreversible damage from the point of view of the immune staining, as this results in a change to the protein-based antigens and this can lead to the sample being unusable.

Another significant disadvantage of the solution is that in the paraffin block poured in two phases, due to the shear forces occurring when producing the section the paraffin block may come apart at the border between the two layers made at two separate points in time, as a result of which the paraffin block may separate from the block holder so making it impossible to make the desired sections from the tissue cylinders.

Examples suitable for the production of paraffin blocks used for the production of tissue samples can also be found in patent specification registration number US 6.383.801 and furthermore, in publication documents numbers WO 01/98525, WO 99/44063, WO 01/22086 and WO 01/42796.

The objective of our invention is to eliminate the disadvantages of the above mentioned methods and to design a tissue micro-array preparing manual apparatus set that is simple, cheap, and compatible with all widely used histological laboratory fixed and semi-fixed assets. The principle of applying the manual apparatus is that the preparation of the recipient block is separated from the sampling; therefore, the samples can be previously prepared and stored elsewhere, then put into the recipient block. The manual apparatus requires small space, is easy to clean, there is basically no need for maintenance, each part is resistant to external physical and chemical impacts, and the recipient block can be prepared in one step.

Our invention is based on the recognition that the tissue micro-array preparing manual apparatus set should be made from elements that fit together in their size and can be applied along with the instruments used under the traditional routine histological laboratory conditions. This tissue micro-array preparing manual apparatus set was developed to contain a tissue core punch extractor and a TMA block mould with lower and upper parts, and the traditional, most frequently used paraffin block holders fit well to this latter one. The tissue core punch extractor is a well-known technical solution, i.e. its function is based on punching. The punching tip is made as a punching tube with an internal diameter of 1 or 2 mm. This punching tube receives the tissue cylinder (core) taken from a given place of the sample (donor block), which can be manually pushed out from the punching tip by help of a piston-like needle probe pushed back by a coil spring inside the instrument. The external diameter of

the needle probe is comparable to the internal diameter of the punching tip, but due to its size it can freely slide within the punching tip.

The TMA block mould is made up of two main elements: the lower and upper parts. The lower part is equipped with 1 or 2 mm wide mandrels, identical with the diameter of the punching tube, organized into lines and columns. Since the lower and upper parts are assembled together during the casting of the paraffin block and the paraffin is poured into the hole formed by the upper part, a margin should have been prepared there, which is identical in its size and shape with the size and shape of the generally used paraffin block holder.

The formation of the margin creates the opportunity to fix the paraffin block holder itself to the empty paraffin block in one step when moulding the paraffin block. This, later on, makes it unnecessary to carry out the subsequent heat treatment, which is essential in the known procedures, and so overcomes the disadvantageous consequences presented above deriving from the heat treatment.

At the lower part at least two vertical directing nipple are built into the base unit, which ensures the parallel and intact removal of the paraffin block when the mould manual apparatus is taken apart. The equal withdrawal of the two elements at the opening of the lower and upper part of the TMA block mould is ensured by at least two diagonally arranged threaded arms.

The elimination of the above discussed disadvantages is ensured by a tissue micro-array preparing manual apparatus set with the following characteristics: a by itself already known tissue core punch extractor and a TMA block mould, which latter has a lower and upper part; the lower part is organized in the middle into lines and columns, set up with mandrels at right angles to the base unit; the upper part is prepared with a margin-containing cleavage, which receives the mandrels when the TMA block mould is assembled together; the margin and size of the cleavage fit to the shape and size of the paraffin block holder; the lower and upper parts are connected to each other with directing nipples and threaded arms fixed horizontally; the lower part of the TMA block mould has at least two vertical directing nipples, which vertically detachably join into the bore-holes of the upper part; in the upper part of the TMA block mould there are at least two diagonal transit bore-holes, which receive the threaded arms and the endings of the threaded arms sit on the lower part when the TMA block mould is assembled together.

Our invention, the TMA building manual set is presented in detail by means of drawings.

Figure 1. Axonometric delineation of the TMA block mould.

Figure 2. Axonometric delineation of the TMA block mould and paraffin block holder of the TMA building manual set.

Figure 3. The finished paraffin block with the tissue core punch extractor.

The 1 TMA block mould shown on Figure 1. is made up of two major elements, i.e. 2 lower part and 3 upper part. The 2 lower part has 4 mandrels with 1 or 2 mm diameter arranged into lines and columns. The diameter of the 4 mandrel is identical with the diameter of the 8 punching tube of the 14 tissue core punch extractor. On the 2 lower part there are 5 directing nipples at right angles to the lower part, which ensure the parallel and intact removal of the paraffin block when the 1 TMA block mould is dismantled. The size of the 3 upper part is identical with that of the 2 lower part and in the middle it has a 7 margin-containing 6 cleavage, which receives the 4 mandrels. The 7 margin of the 6 cleavage is made to receive the commercially available 12 paraffin block holder shown on Figure 2. On the 2 lower part there are 5 directing nipples, which join the 10 transit bore-holes on the 3 upper part. Moreover, the additional 11 transit bore-holes on the 3 upper part receives the 9 threaded arms, which ensure the even and parallel opening of the two parts of the assembled instrument cast with paraffin block.

The equal withdrawal of the two elements at the opening of the 2 lower and 3 upper part of the 1 TMA block mould is ensured by at least two diagonally arranged 9 threaded arms. The 14 tissue core punch extractor ends in a 8 punching tube with an internal diameter of 1 or 2 mm.

The well-known 14 tissue core punch extractor receives the tissue cylinder taken from a given place of the sample (donor block), which can be manually pushed out from the 8 punching tube by help of a piston-like needle probe pushed back by a coil spring inside the instrument.

The external diameter of the needle probe is comparable to the internal diameter of the 8 punching tube, but due to its size it can freely slide within the 8 punching tip.

On preparing the paraffin block, the assembled 1 TMA block mould is filled with paraffin through the 6 cleavage. After the paraffin is set when cooled down and removed from the instrument, it gives a 13 paraffin block (recipient block) containing 1 or 2 mm wide cylindrical holes with parallel longitudinal axes organized into regular lines and columns. These holes receive the tissue cores having the same diameter taken out from the donor blocks by the 14 tissue core punch extractor, giving finally the TMA block.

As it can be seen from our description, our objective was accomplished by the development of our invention. The TMA building manual set is a simple, cheap manual instrument, which is easy to handle and clean, environment-friendly, and enables the fast and accurate preparation of TMA under the traditional, routine histological laboratory conditions.

A further advantage is that due to the unique formation of the manual set it is possible to produce the paraffin block with holder in one step without any subsequent heat treatment, which does not only reduce the time required for the production but also has a favourable effect on the mechanical characteristics of the finished piece, that is also including the shearing strength.

-Claims

1. The tissue micro-array (TMA) building manual set, which includes a well-known tissue core punch extractor (14) and a TMA block mould (1), the TMA block mould (1) has a lower part (2), where this lower part (2) has mandrels (4) at right angles to the base unit arranged in the middle into lines and columns, **characterized** with the TMA block mould (1) is supplemented with an upper part (3) the upper part (3) has a margin-containing (7) cleavage (6), which receives the mandrels (4) when the TMA block mould (1) is assembled, the margin (7) and size of the cleavage (6) fit to the shape and size of the paraffin block holder (12), and the lower (2) and upper (3) parts are connected to each other with directing nipples (5) and threaded arms (9) fixed horizontally.
2. The manual apparatus set as in the claim point No. 1 is characterized with: the lower part (2) of the TMA block mould (1) has at least two vertical directing nipples (5), which join the bore-holes (10) of the upper part (3) and are in vertical direction freely movable.
3. The manual apparatus set as in the claim point No. 1 is characterized with: the upper part (3) of the TMA block mould (1) has at least two diagonal transmit bore-holes (11), which receive the threaded arms (9) sit on the lower part (2) when the TMA block mould (1) is assembled together.

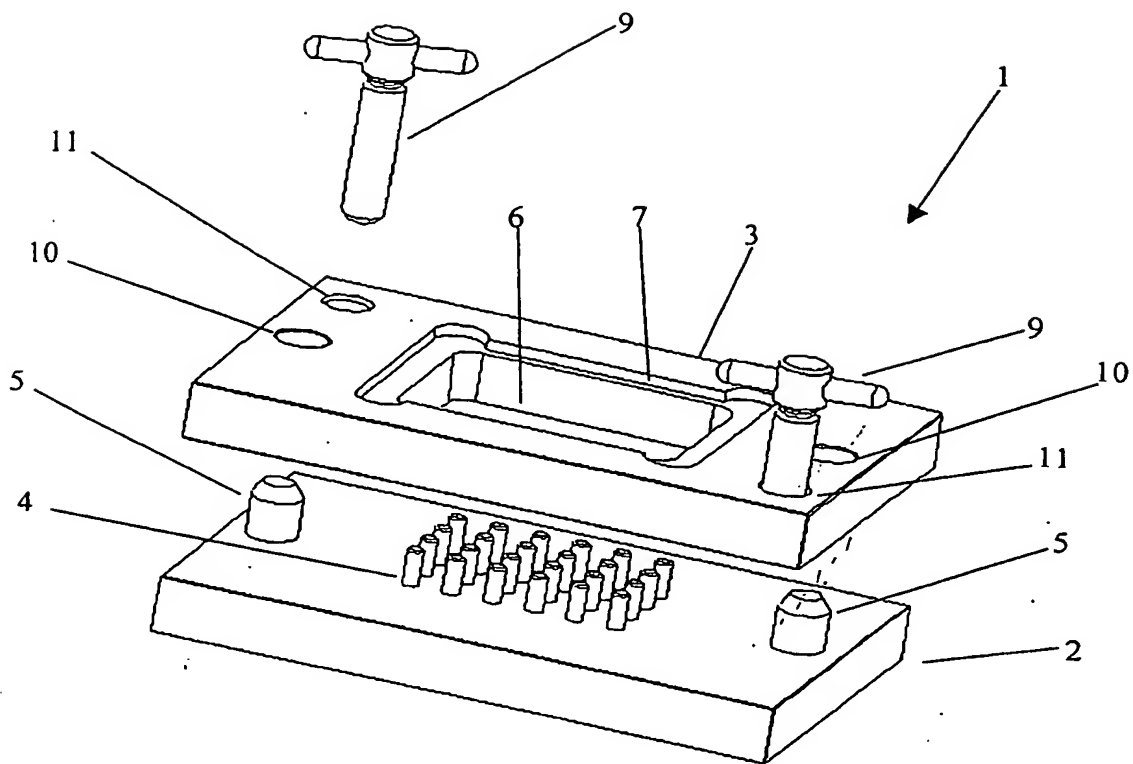


Figure 1

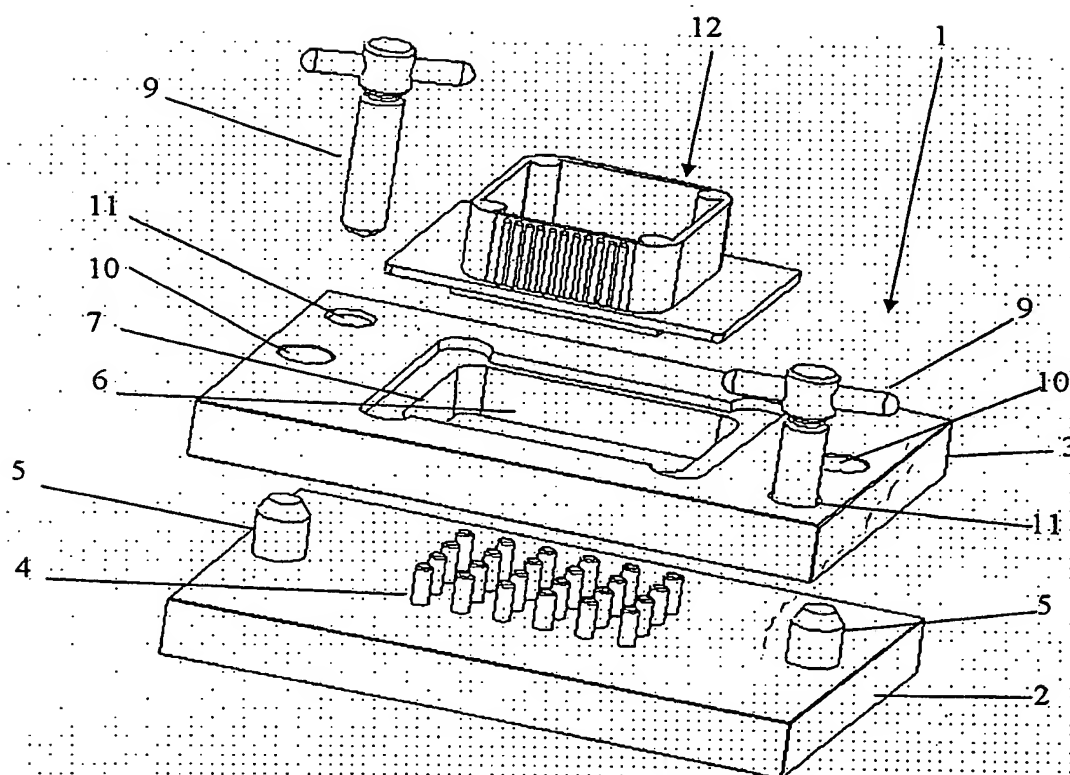


Fig. 2

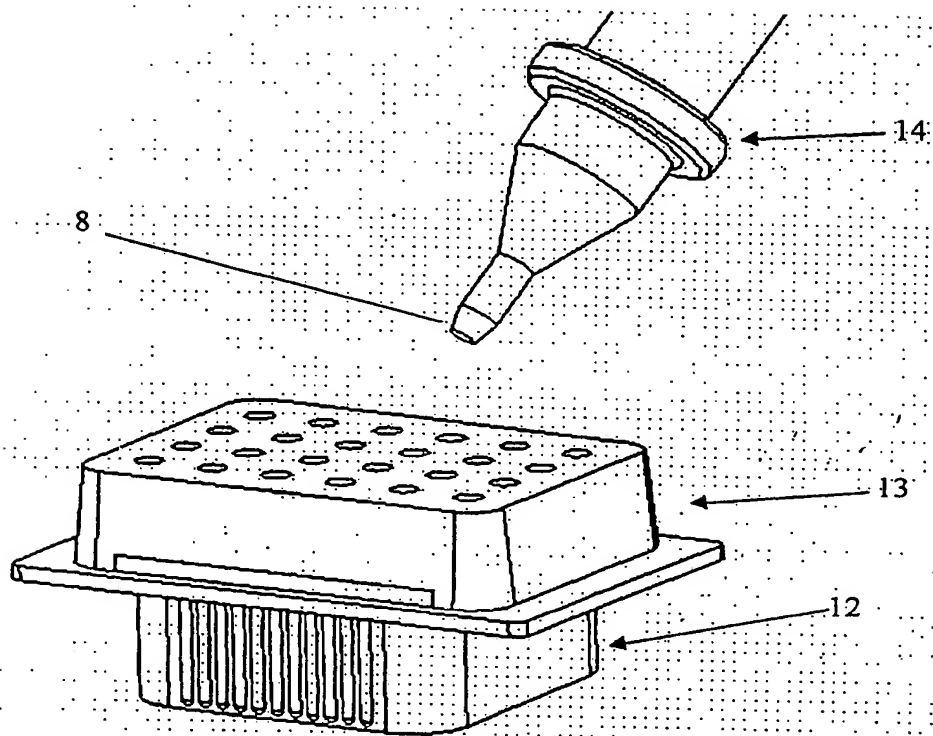


Fig. 3

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